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The Foragining Ecology of the Green Turtle in the Baja California Peninsula: Health Issues

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1. Introduction

Conservation of threatened species, such as the green turtle (*Chelonia mydas*), is closely related to habitat quality. In particular there are issues related to heavy metals, the presence of epibionts, parasites and fibropapiloms who might play a crucial role in the species survivorship. Heavy metals occur naturally in the environment (Sparling et al., 2000) as part of the biogeochemical cycles (Valiela, 2009), and it is often difficult to differentiate between natural and anthropogenic sources (Kieffer, 1991; Moreno, 2003). In marine systems, natural processes (e.g., upwelling, river runoff) can redistribute and concentrate heavy metals in the environment, occasionally reaching toxic levels (Sparling et al., 2000; Machado et al., 2002). The effects of these processes may vary over seasonal and spatial scales (Sawidis et al., 2001) and their understanding can aid in determining the sources as biomonitors (Szefer et al., 1998; Páez-Osuna et al.,2000), and ultimately their effects on wild life (Sparling et al., 2000; Talavera-Saenz et al., 2007). Also, they can be used for bioabsorption in contaminated waters (Kumar and Kaladharan, 2006). Caliceti et al. (2002) found a decrease in Zinc and Cadmium concentrations from the center of a lagoon, close to an industrial district, towards the Venice lagoon (Italy) openings to the sea, suggesting anthropogenic sources, while Villares et al. (2002) found that seasonal and spatial variation in metals was related to algal growth cycles and river runoff. Riosmena-Rodriguez et al. (2010) determined that heavy metals are related to the physiological features of each major analyzed taxon (green algae, red algae and seagrasses).

The processes controlling the concentration and distribution of metals in coastal environments and their consequences in the species health are poorly understood. It is generally assumed that diet is the main source of metals to sea turtles (Caurant et al., 1999; Anan et al., 2001), but little is known of the process of metal accumulation in these species because data on metal residues in most components of sea turtles' diet has been lacking. As adults, green turtles forage largely on marine algae and seagrasses with variation in the diet due to the relative availability of food types over geographic and temporal scales (Garnett et al., 1985; Brand-Gardner et al., 1999; Seminoff et al., 2002). In the process of metal bioaccumulation in marine food chains is poorly understood because very little data is available on metal concentration at different trophic levels (de la Lanza et al. 1989; Talavera-Saenz et al. 2007) or their temporal (Abdallah et al., 2006; Rodriguez-Castañeda et al., 2006) or spatial variation (Kalesh and Nair, 2006) and their effects on the photosynthetic process (Catriona et al. 2002). High concentrations of heavy metals have been found in sea turtles from many regions of the world (Storelli and Marcotrigiano, 2003). Although metal concentrations vary greatly by region and tissue type, green turtles (*Chelonia mydas*) have been found to have exceptionally high kidney cadmium concentrations. Elevated Cadmium levels have been measured in green turtles from around the world including Japan (Sakai et al., 2000; Anan et al., 2001), China (Lam et al., 2004), Europe (Caurant et al., 1999), Australia (Gordon et al., 1998) and the Arabian Sea (Bicho et al., 2006). Gordon et al. (1998) found that Cadmium concentrations in green turtles from Australia were up to three times higher than the levels reported in commercial seafood products. The presence of epibionts, parasites (internal and external) might occasionally cause the death of some marine turtles and being predecessors of fibropapiloms (Aguirre y Lutz, 2004; Work, 2000, Work *et al*., 2005). The presence of fibropapiloms in Hawaiian waters was related with the presence of hirudineans (Díaz, *et al.,* 1992). This kind of infections are might be related with their foraging habitat and its conservation condition, their health condition to escape predators and, for the females, the fecundity reduction (Gámez et al., 2006; Alfaro, et al., 2006; Badillo, 2007).

The Baja California Peninsula serves an important role as foraging grounds for five of the world's seven sea turtle species (Gardner and Nichols, 2001). Although much of the peninsula is considered pristine, exploitation of mineral deposits has occurred since the 19th Century and concentrations of Cadmium, Zinc, Copper and Plumb in sediment and marine fauna have been observed above those in more industrialized regions (Gutiérrez-Galindo et al., 1999; Shumilin et al., 2000). In the mid 1970's, Martin and Broenkow (1975) reported that concentrations of Cadmium along the coast of the Baja California Peninsula were remarkably elevated as compared to other regions of the eastern Pacific. Sources of heavy metals in Baja California have been generally attributed to natural factors related to upwelling and the biogeochemistry of the region, however, the potential contribution from anthropogenic sources (e.g. mining and urbanization) cannot be entirely dismissed (Martin and Broenkow, 1975; Sañudo-Wihelmy and Flegal, 1996; Méndez-Rodríguez et al., 1998; Gutiérrez- Galindo et al., 1999; Shumilin et al., 2001). Rodríguez-Meza et al. (2008) developed an extensive evaluation of the heavy metals in sediments and seaweeds along ten sites in the bay. They suggested that the high levels of some heavy metals are related to terrigenous input from the arroyos and biogenic origin by the upwelling. In order to better understand the sources of heavy metals to marine species, more information is needed on metal concentrations in primary producers that make up the base of the food chain. However, few (Riosmena-Rodriguez et al., 2010) papers have approached the study of natural levels of heavy metals in seaweed communities and their temporal and spatial variation. Previous studies in Magdalena Bay, Mexico (Méndez et al., 2002; Gardner et al., 2006) have found high concentrations of metals in marine vertebrates, despite the lack of obvious anthropogenic sources. For example, Cadmium, Zinc, and Iron concentrations in the herbivorous green turtle, *Chelonia mydas*, were the highest ever reported in sea turtles globally (Gardner et al., 2006). In Magdalena Bay, like other regions of the Baja California

Peninsula (Seminoff et al., 2002), juvenile and adult green turtles preferentially consume soft red algae, especially species of Gracilaria (López-Mendilaharsu et al., 2005). Studies in Baja California have demonstrated that these same species of red algae tend to have higher enrichment factors of metals than other groups of seaweeds (Sánchez-Rodríguez et al., 2001), which could account for the high accumulation of metals in foraging green turtles in this region.

2. Materials and methods

2.1 Study area

Magdalena Bay is located on the Pacific coast of the Baja California Peninsula, Mexico between 24° 15′ N and 25° 20′ N, and 111° 30′ W and 112° 15′ W. It is a shallow lagoon protected from the Pacific by barrier islands, with high productivity resulting from seasonal marine upwelling along the coast. Diverse marine habitats within the bay include sandy bottoms and rocky margins, extensive beds of the seagrass *Zostera marina* and a diverse assemblage of macroalgae. A sea turtle refuge area known as Estero Banderitas is located within the mangrove channels in the northwest region of the Bay where green turtles reside year-round (Fig. 1). Because of the perceived importance of this area for green turtle foraging, its protection has been identified as a priority for conservation efforts (Arriaga et al., 1998; Nichols et al., 2000). Rodríguez-Meza et al. (2008) has found that the presence of heavy metals in the bay is heavily influenced by sediment type, organic material, and carbonates and concluded that there was no evidence of human impacts.

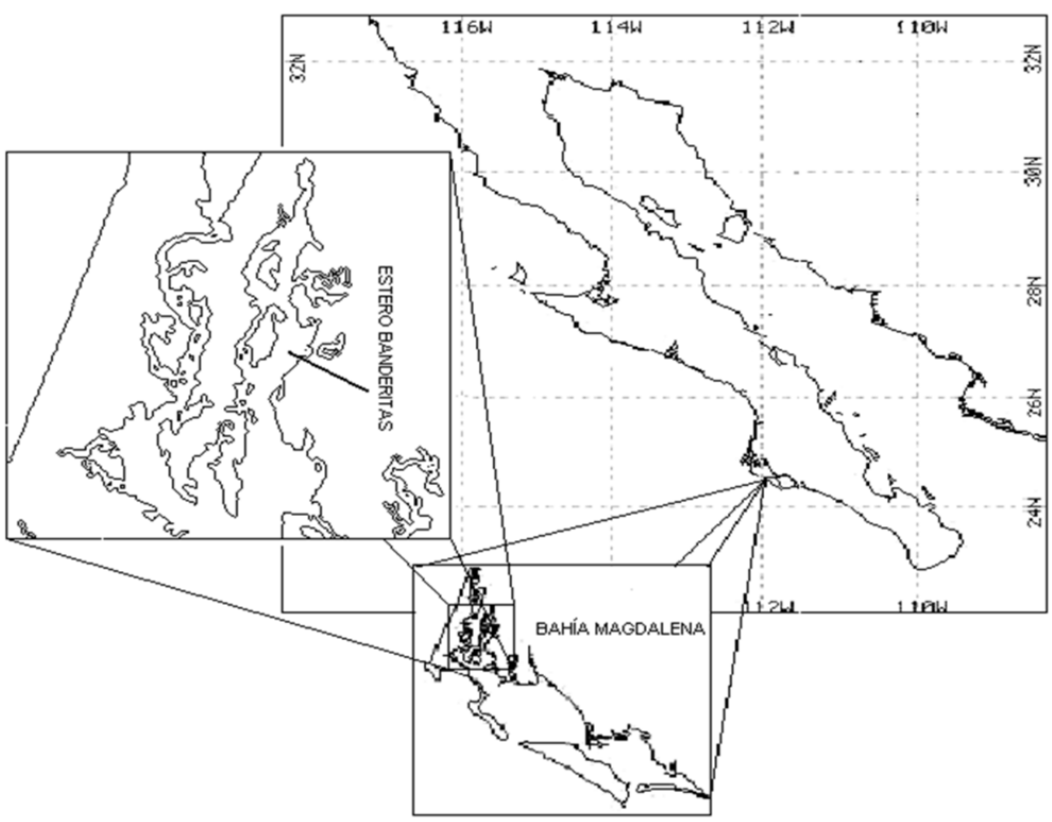

Fig. 1. Study area in Estero Banderitas (24º 50´ - 25º 00´ N and 112º08´ W) located in Bahía Magdalena, Baja California Sur, Mexico.

2.2 Marine plant collection

Three separate sampling trips were made in Estero Banderitas (November 2004, February, 2005 and April, 2005) in order to collect marine plants available during different seasons. Algae and seagrass samples were collected along the length of the mangrove channel using 16 transects of 30 m length. Every 6 m along the transects, plants were manually collected within a 25 cm2 to 1 m2 area, depending on the density of the flora at that location, for a total of 80 samples per trip. The samples were stored in labeled plastic bags and contents were separated by species using taxonomic keys (Riosmena Rodríguez, 1999). Samples were sun-dried in the field and then pressed to further remove moisture.

2.3 Sea turtle tissue collection

Liver and kidney tissues were collected from 8 dead green turtles that incidentally drowned in commercial fishing nets set in Magdalena Bay between February 2002 and April 2003. The straight carapace length of the turtles ranged from 47–77 cm, which is representative of the size range of green turtles in the region (Gardner and Nichols, 2001). The samples were collected within 24 h after the time of death from carcasses with minimal decomposition. Tissue samples were stored in plastic bags and placed on ice for transport to the laboratory where they were frozen at − 80°C until analyzed. From five turtles, intact stomachs were also collected.

2.4 Stomach content analyses

All stomach contents were collected and identified to the lowest possible taxonomic level based on published keys (Abbott and Hollenberg, 1976; Riosmena-Rodríguez, 1999). Entire sample volume and the relative sample volume of each plant species were calculated by the procedure of water displacement in a graduated cylinder. Voucher material was housed in Herbario Ficológico of the Universidad Autónoma de Baja California Sur (UABCS), La Paz, México.

2.5 Laboratory analyses

Tissue and plant samples (0.5 g) were dried in an oven at 70 °C until a dry weight was obtained. Dried samples were digested in acid-washed Teflon tubes with concentrated nitric acid in a microwave oven (CEM modelMars 5X, Matthews, NC). Samples were analyzed by atomic absorption (GBC Scientific equipment, model AVANTA, Dandenong, Australia) using an air-acetylene flame. The certified standard reference material, TORT-2 (National Research Council of Canada, Ottawa) was used to verify accuracy, and that the analytical values were within the range of certified values. All recoveries of metals analyzed were over 95%. Detection limits were: Zinc=0.0008, Cadmium = 0.0009, Mn= 0.002, Cu= 0.0025, Ni = 0.004, Fe=0.005, Pb=0.006 μg/g.

2.6 Quantitative analyses

We analyzed the data based on taxonomic group (red algae, green algae, and seagrass), season, spatial area, and dominant species. Reported statistics are medians (nN2) and ranges in μg/g on a dry weight basis. The Mann–Whitney test was used for conducting two-tailed sample comparisons of tissues for each metal separately and for comparing metals in marine plants collected in Magdalena Bay with those found in the stomach contents. The Kruskal–Wallis test was used to compare the median metal concentration across all plant species. The null hypothesis was rejected if $p \leq 0.05$. The influence of concentration differences among samples was removed by converting data to the percent contribution of each metal to the total metal signature of the individual sample. Fe was removed from these analyses because of its high Concentration and dominance of the metal signature profile. Principal Components Analysis (PCA) of the percent contribution of the metals in plants and turtle tissues. Additionally, factorial analysis was used to determine trends in the presence of heavy metals in the seaweed samples and the relative spatial and/or temporal variation. All analysis was conducted using the Statgraphics Plus software program (Version 5, Rockville, MD).

2.7 Prescence of fibropapiloms and epibionts

Monthly sampling has been develop in the Estero Banderitas and more recently in Estero San Buto as part of the monitoring efforts in was the prescence of fibropapiloms and epibionts by a physical inspection of each animal by region as head, neck, carapace, front or back fins, anus or tail. Comparative analysis was done of the proportion of animals with fibropapiloms and epibionts using the database and literature described in Lara-Uc (2011) in relation to the Bahía Madalena population information (Hinojosa-Arango unpublished data).

3. Results

3.1 Temporal and spatial variation of metal concentration in plant species

Based on our analysis, we found temporal and spatial variations in the concentration in several of heavy metals in seaweeds and seagrasses. In comparisons between the profiles of heavy metals in major plant groups, we found that Nickel differed significantly between the major groups ($P=0.01$), wherein seagrasses had lower concentrations (Tables 1 and 2). Analyzing all of the species (all sites combined), we found significant seasonal differences in the heavy metal concentrations with the exception of Zinc ($P=0.53$). Samples collected in April had a higher concentration of Cadmium ($P<0.001$) and Iron ($P=0.002$) and a lower concentration of Plumb ($P<0.001$) and Nickel ($P=0.002$) than the other months. Manganese was highest in November ($P=0.049$) and Copper was higher in November compared to February ($P=0.01$). In comparisons of the metal concentrations between plant species, the only significant differences were detected for Cadmium ($p=0.009$) in *Ruppia maritima* than all other species. In the case of the analysis of green algae alone, using all species combined, we found temporal significant differences of Cadmium in April ($P=0.01$).

In the case of other metals, we found significantly temporal differences in Plumb (Pb) concentration in *G. vermiculophylla* ($P=0.02$) in November but this species also had the highest concentration of Ni ($P=0.03$) in relation to the other species. Also, there were significant differences in the concentrations of Cadmium ($P=0.001$), Iron ($P=0.01$), and Nickel ($P=0.002$), while Plumb ($P<0.001$) and Copper ($P=0.03$) were significantly different than the same metals in November. In the same month, highest Nickel concentrations were recorded in *Codium amplivesiculatum*, while in April, *C. amplivesiculatum*, *Codium cuneatum*, and *Caulerpa sertularioides* from the middle region had the highest concentrations of Copper (7.3μg g^{-1} dw), Ni (11μg g^{-1} dw), and Mn (61.4μg g^{-1} dw), respectively. In February, like November, we had the highest Iron concentration and several species were responsible for this difference (in *H. johnstonii*; 567.5μg g^{-1} dw) and Zinc concentration (in *G. textorii*; 46.8μg g^{-1} dw). However, the lower zone had the highest concentrations of Cadmium (in *G. textorii*; 4.4μg g^{-1} dw) and Ni (*L. pacifica* and *Chondria nidifica*; 13.3 and 13.3μg g^{-1} dw). Copper (in *L. pacifica*; 2.9μg g^{-1} dw) and Plumb concentrations were highest in *G. andersonii* from the middle zone (3.8μg g^{-1} dw).

Season	Species	Cadmium	Plumb	Nickel	Manganese	Iron	Copper
November	*Codium amplivesiculatum*	0.2 (nd - 0.5)	1.8 (1.3 - 2.3)	8 (6 - 9.9)	52.9 (42.2 - 63.5)	362.2 (349.8 - 374.7)	0.9 (0.7 - 1.2)
	Gracilaria textorii	1.5 (nd - 3.9)	1.4 (nd - 1.9)	4.8 (3 - 5.1)	48.5 (45.3 - 51.1)	325 (100.9 - 1231.2)	1.6 (0.7 - 4.8)
	Gracilaria vermiculophylla	0.6 (0.5 - 1.4)	2.7 (1 - 3.3)	5.3 (4.9 - 5.5)	22.4 (13 - 23.9)	302.7 (185.9 - 372.2)	1.3 (1 - 1.6)
	*Gracilariopsis andersonii**	0.5 -	2 -	5.7 -	28.5 -	195.2 -	2.5 -
	Hypnea johnstonii	0.4 (0.3 - 1.5)	1.8 (1.1 - 8.5)	6.7 (6 - 6.9)	26.7 (23.7 - 282.5)	263.9 (227.8 - 1424.1)	1.8 (0.9 - 4.4)
February	*Codium amplivesiculatum*	nd	0.8 (0 - 2.3)	6.6 (6.2 - 7.3)	12.6 (12.1 - 20.4)	190.2 (189.5 - 522.7)	0.8 (nd - 1.3)
	*Codium cuneatum**	nd -	1.6 -	5.9 -	17.2 -	241.7 -	0.4 -
	Chondria nidifica	1 (nd - 1.7)	1.6 (1.5 - 1.6)	9.3 (5.1 - 13.3)	15.6 (14.40- 21)	291.5 (88.8 - 557.8)	1.3 (0.2 - 1.4)
	Gracilaria textorii	3.4 (2.7 - 4.4)	1 (0.7 - 2)	6 (4.5 - 6.2)	49.1 (43.5 - 54.8)	139.9 (81.8 - 476.3)	0.5 (0.4 - 1.2)
	Gracilaria vermiculophylla	1.1 (1.1 - 1.6)	0.8 (0.7 - 0.9)	4.3 (3.6 - 5.1)	19.3 (14.4 - 19.5)	206.2 (139.4 - 269.9)	0.6 (0.3 - 1.6)
	*Gracilariopsis andersonii**	1.6 -	3.8 -	4.5 -	23.5 -	160.4 -	2.1 -
	*Hypnea johnstonii**	nd -	nd -	11.3 -	20.6 -	567.5 -	nd -
	*Laurencia pacifica**	3 -	1.7 -	13.3 -	25.2 -	195.8 -	2.9 -
	*Sarcodiotheca gaudichaudii**	0.9 -	1 -	5.4 -	17.2 -	121.8 -	0.1 -
	*Zostera marina**	nd -	2.5 -	3.1 -	78.6 -	51.1 -	0.4 -
April	*Codium amplivesiculatum*	1.6 (1.2 - 1.9)	0.5 (0.4 - 0.7)	7.8 (7.6 - 7.9)	18.7 (15.3 - 22.1)	399.2 (298.1 - 500.4)	4.1 (1 - 7.3)
	Codium cuneatum	2.1 (1.9 - 2.2)	0.3 (0.1 - 0.5)	7.1 (3.2 - 11)	16.7 (10.5 - 23)	284.3 (141.5 - 427.1)	1.2 (0.5 - 1.8)
	Caulerpa sertularioides	2.1 (1.8 - 2.3)	0.2 (nd - 0.4)	2.6 (1.8 - 3.4)	34.3 (7.3 - 61.4)	374 (223.9 - 524.1)	1.8 (1.1 - 2.6)
	*Gracilaria crispata**	4.6 -	nd -	3.9 -	40.3 -	576.8 -	1.6 -
	Gracilaria textorii	4.5 (4.3 - 4.8)	0.4 (0.1 - 0.6)	5.3 (3 - 7.6)	41.5 (37.6 - 45.4)	579.5 (578.4 - 580.6)	1.7 (1.5 - 1.8)
	Gracilaria vermiculophylla	2.9 (2.7 - 2.9)	0.2 (nd - 0.6)	2.9 (1.1 - 2.9)	18.1 (14.7 - 23.6)	236.2 (214.4 - 771.5)	0.9 (0.9 - 1.6)
	*Gracilariopsis andersonii**	3.8 -	0.1 -	2.3 -	25.5 -	322.3 -	1.5 -
	*Hypnea johnstonii**	2.7 -	0.6 -	1.8 -	41.9 -	774.5 -	2.1 -
	*Laurencia pacifica**	4.6 -	nd -	1.9 -	22.9 -	497.6 -	1.8 -
	Ruppia maritima	4.5 (2.1 - 7)	2.1 (0.5 - 3.8)	2.3 (1.7 - 2.9)	30.6 (28.6 - 32. 6)	1230.2 (1017.4 - 1443)	0.5 (nd - 0.9)
	*Zostera marina**	2.2 -	nd -	2.8 -	33.9 -	630.3 -	1.6 -

* The values are referred to 1 specimen. nd signifies not detected.

Table 1. Temporal variation of heavy metal concentrations µg.g-1 dry weight in seaweeds and seagrasses collected at the Estero Banderitas. Values are expressed as medians and ranges given in parenthesis.

Site	Species	Cadmium	Plumb	Nickel	Manganese	Iron	Copper
Head	*Codium amplivesiculatum**	nd	2.3	6.6	20.4	522.7	0.8
		-	-	-	-	-	-
	*Chondria nidifica**	nd	1.5	5.1	14.4	88.8	0.2
		-	-	-	-	-	-
	Gracilaria textorii	1.3	1	4.5	50.1	853.7	3
		(nd - 2.7)	(nd - 2)	(3 - 6)	(45.3 - 54.8)	(476.3 - 1231.2)	(1.2 - 4.8)
	Gracilaria vermiculophylla	1.1	0.7	5	22.4	236.2	0.8
		(0.6 - 2.9)	(0.2 - 3.3)	(1.1 - 5.1)	(19.5 - 23.6)	(206.2 - 372.2)	(0.3 - 1.6)
	*Gracilariopsis andersonii**	0.5	2	5.7	28.5	195.2	2.5
		-	-	-	-	-	-
	Hypnea johnstonii	0.7	4.3	9.1	151.6	995.8	2.2
		(nd - 1.5)	(0 - 8.5)	(6.9 - 11.4)	(20.6 - 282.5)	(567.5 - 1424.1)	(nd - 4.4)
	*Ruppia maritima**	6.9	3.8	2.9	32.6	1017.4	nd
		-	-	-	-	-	-
Medium	*Coduim amplivesiculatum*	0.5	0.7	7.9	22.1	349.8	1.2
		(nd - 1.2)	(nd - 1.3)	(7.3 - 10)	(12.1 - 63.5)	(190.2 - 500.4)	(nd - 7.3)
	Codium cuneatum	0.9	1	8.4	20.1	334.4	1.1
		(nd - 2.3)	(0.5 - 1.6)	(5.9 - 11)	(17.2 - 22.9)	(241.7 - 427.1)	(0.4 - 1.8)
	*Caulerpa sertularioides**	2.3	0.4	3.4	61.4	524.1	2.5
		-	-	-	-	-	-
	*Chondria nidifica**	1	1.6	9.3	20.9	557.8	1.4
		-	-	-	-	-	-
	Gracilaria textorii	3.9	1	4.8	45.4	325	0.7
		(3.4 - 4.8)	(0.6 - 1.4)	(4.5 - 7.6)	(43.5 - 51.2)	(139.9 - 580.6)	(0.4 - 1.8)
	Gracilaria vermiculophylla	1.4	0.9	3.5	18.1	302.7	1.4
		(1.1 - 1.6)	(0.6 - 1)	(2.9 - 5.5)	(13 - 19.3)	(269.9 - 771.5)	(1 - 1.6)
	*Gracilariopsis andersonii**	1.6	3.8	4.5	23.5	160.4	2.1
		-	-	-	-	-	-
	*Hypnea johnstonii**	0.3	1.1	6.7	26.7	263.9	1
		-	-	-	-	-	-
	*Ruppia maritima**	2.1	0.5	1.7	28.6	1443	0.9
		-	-	-	-	-	-
	*Sarcodiotheca gaudichaudii**	0.9	1	5.4	17.2	121.8	0.1
		-	-	-	-	-	-
Mouth	*Codium amplivesiculatim*	nd	0.8	6.2	15.3	298.1	1
		(nd - 1.9)	(0.4 - 2.3)	(6 - 7.6)	(12.6 - 42.2)	(189.5 - 374.7)	(0.7 - 1.3)
	*Codium cuneatum**	2.2	0.1	3.2	10.5	141.5	0.5
		-	-	-	-	-	-
	*Caulerpa sertularoides**	1.8	nd	1.8	7.3	223.9	1.1
		-	-	-	-	-	-
	*Chondria nidifica**	1.7	1.6	13.3	15.6	291.5	1.3
		-	-	-	-	-	-
	*Gracilaria crispata**	4.6	nd	3.9	40.3	576.8	1.6
		-	-	-	-	-	-
	Gracilaria textorii	4.3	0.7	5.1	48.5	100.9	1.5
		(1.5 - 4.4)	(0.1 - 1.9)	(3 - 6.2)	(37.6 - 49.1)	(81.8 - 578.4)	(0.5 - 1.6)
	Gracilaria vermiculophylla	1.6	0.8	4.3	14.7	186	0.9
		(0.5 - 2.9)	(0 - 2.7)	(2.9 - 5.3)	(14.4 - 23.9)	(139.4 - 214.4)	(0.6 - 1.3)
	*Gracilariopsis andersonii**	3.8	0.1	2.3	25.5	322.3	1.5
		-	-	-	-	-	-
	Hypnea johnstonii	1.6	1.2	3.9	32.8	501.1	1.9
		(0.4 - 2.7)	(0.6 - 1.8)	(1.8 - 6)	(23.7 - 41.9)	(227.8 - 774.5)	(1.8 - 2.1)
	Laurencia pacifica	3.8	0.8	7.6	24	346.7	2.3
		(3 - 4.6)	(nd - 1.7)	(1.9 - 13.3)	(22.9 - 25.2)	(195.8 - 497.6)	(1.8 - 2.6)
	*Zostera marina**	2.2	nd	2.8	33.9	630.3	1.6
		-	-	-	-	-	-

* The values are refered to 1 specimen. nd signifies not detected.

Table 2. Heavy metal concentrations (µg.g-1 dry weight) in seaweeds and seagrasses collected in the three sites. Values are expressed as medians and ranges given in parenthesis.

Spatial differences in metal concentrations were dependent on the major taxa. In the case of seagrasses, we found a high concentration of Iron (Table 2) who was significant different from Manganese (in *Z. marina*; 78.6 µg g−1 dw) concentrations were highest in the upper zone (P=0.01) because their uneven distribution in the area. Consistent with the above analysis were the multifactorial analysis (Fig. 2) wherein the extreme values are represented by Iron and Manganese with no association among seasons or areas. In the green algae (Table 2), we were able to find many metals in the entire area, but the significant difference was found in Cadmium in April (P=0.01), when all species combined, because the low value in relation to other metals are highly concentrated. There is no consistent pattern in relation to the area of the highest concentration of any metal; they tend to present a group lower in relation to higher concentration in different areas or times (Tables 1 and 2).

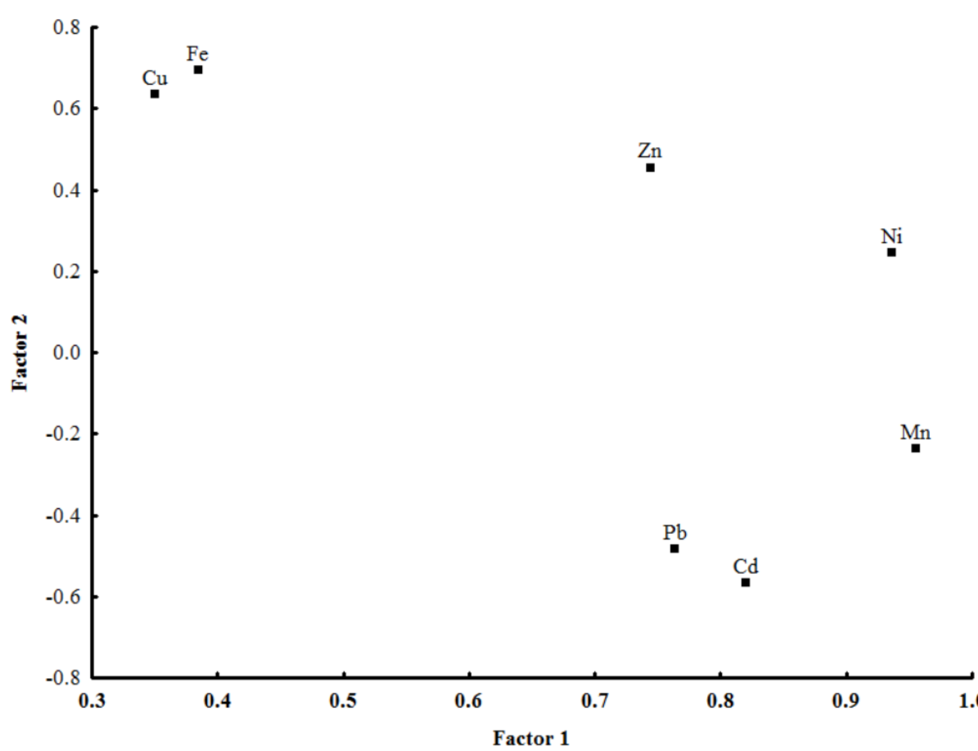

Fig. 2. Multivariate analysis of heavy metals contents in seagrasses.

This is well supported by the multivariate analysis (Fig.3) wherein most of the observed metals show a combination among them and the areas of sampling. We found an extremely high variability in the median content in the red algae (Table 2) but there were no significant differences between sites, with the exception of Zinc which was significantly higher in the upper zone (P=0.02). The highest concentration of any metal was Iron in *Hypnea johnstonii* from the upper zone (1,424.1µg g−1 dw). The highest concentration of Manganese (282.5µg g−1 dw) and Plumb (8.5) µg g−1 dw) were also detected in *H. johnstonii* from the upper zone. Similarly, Zinc (58.8µg g−1 dw) and Copper (4.8µg g−1 dw) concentrations were highest in *G. textorii* in the same zone. The highest Cadmium concentrations were measured in *G. textorii* (4.8µg g−1 dw).

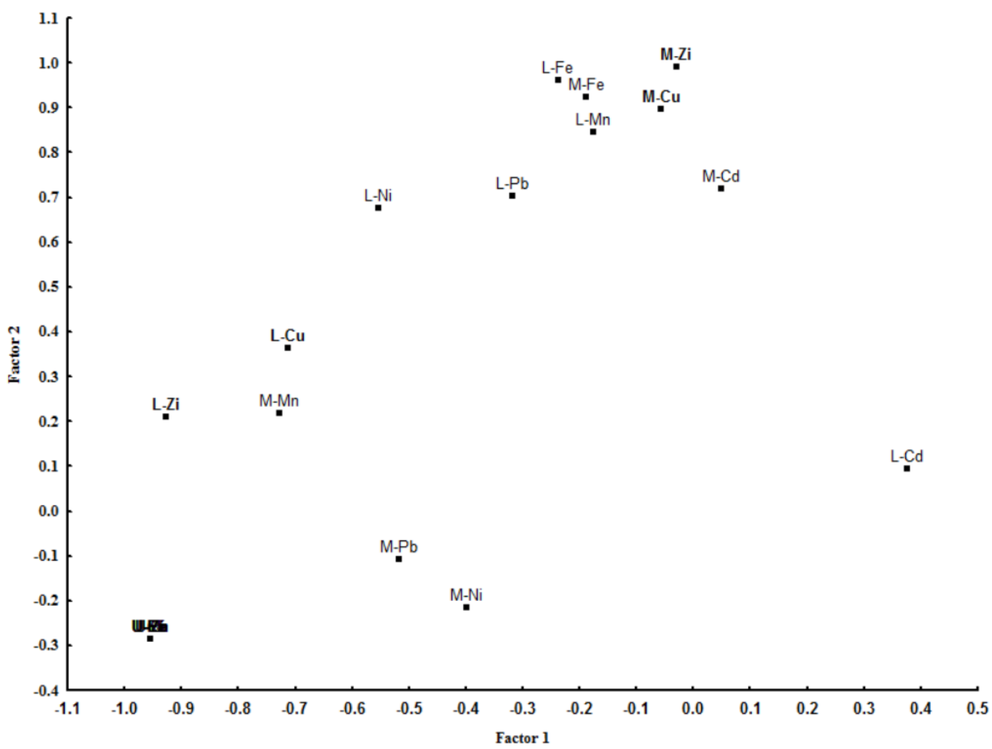

Fig. 3. Multivariate analysis of the spatial concentration of heavy metal in green algae.

Multivariate analyses show the same path in red algae (Figs. 4 and 5) with the clump of areas within metals and a group of metals with high concentration (Fig. 4) in relation to metals with low concentration (Fig. 5).

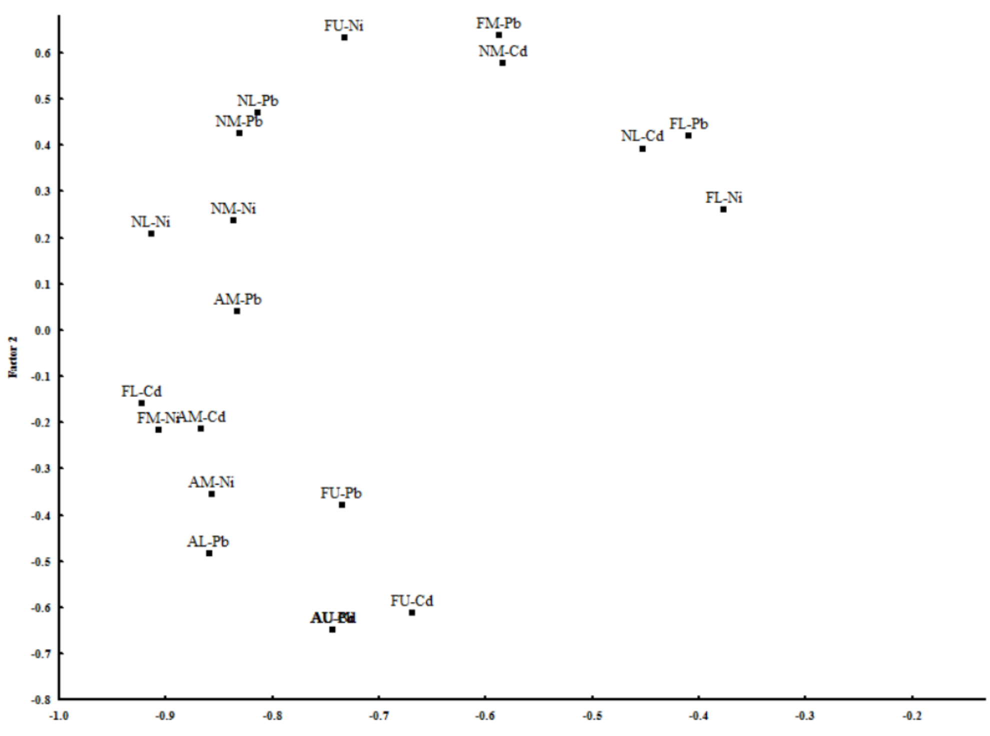

Fig. 4. Multivariate analysis of the spatial concentration of heavy metal in red algae.

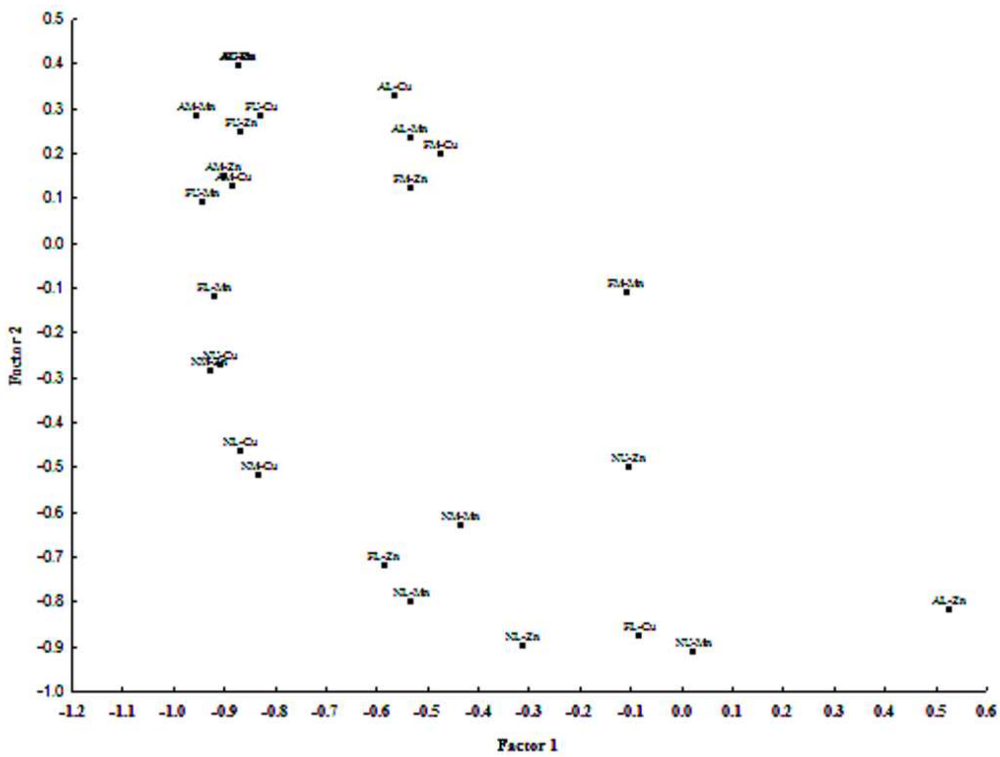

Fig. 5. Multivariate analysis of the spatial concentration of heavy metal in red algae.

3.2 Metals in sea turtle tissues, stomach contents, and plants from the bay

Concentrations of Cadmium and Zinc in flora from the sea turtle stomach contents were greater than the same species of marine plants collected in the bay ($p<0.001$ and $p=0.003$, respectively) (Figure 6). For both metals, the concentrations in sea turtle liver were not significantly different from the stomach contents. Sea turtle kidney Cadmium concentration was significantly higher than liver ($p=0.002$), while Zinc was the same in both tissues. Plumb, Manganese and Fe in flora from the stomach contents were significantly lower than in flora collected from the bay ($p<0.001$ for each) (Figure 6). The stomach contents had higher Plumb and Manganese concentrations than liver ($p=0.04$ and $p<0.001$, respectively) but were not significantly different in Fe. There were no differences in the concentrations of these metals in liver and kidney. Nickel and Copper concentrations did not differ in plants from the two sources. Nickel concentration in liver was similar to kidney concentrations, but significantly lower than the stomach contents ($p=0.005$). Copper was higher in liver than stomach contents ($p<0.001$) and higher than kidney ($p<0.001$). These same trends persisted when the data were transformed to the percent contribution of the metals in each plant species in the stomach contents as compared to the bay samples (Fig. 6). For each of the five plant species, the percent contribution of Manganese and Plumb was greater in the bay-collected plants, while Cadmium and Zinc consistently contributed more to the total metal profile in plants from the stomach contents. Fig. 7 shows the percent contribution of each metal in paired samples of liver, kidney and stomach contents (all flora combined) from the same turtles. Cadmium and Zinc contributed most to the overall metal profile in the kidney, while Copper contributed more in liver. The percent contribution of Manganese and Nickel were greatest in the plants from the stomach contents.

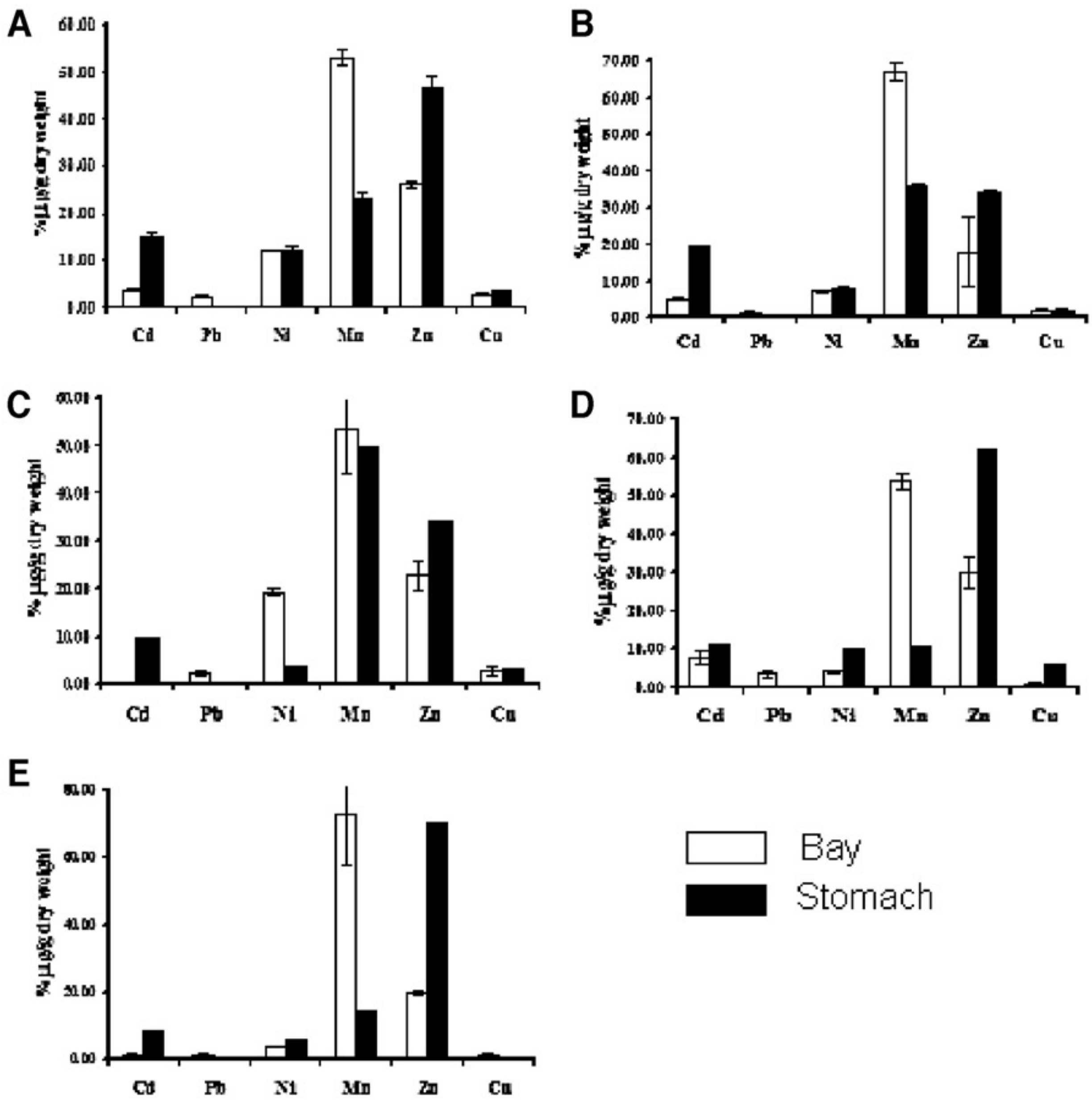

Fig. 6. Percent contribution of metals in species of marine flora collected in the Magdalena Bay and in green turtle (*Chelonia mydas*) stomach contents. A) *G. vermiculophylla*, B) *G. textorii*, C) *C. amplivesiculatum*, D) *R. maritima* and E) *Z. marina*.

Eight species of marine flora were identified within the green turtle stomach contents (Table 3). These same species were also collected from the mangrove channel of Estero Banderitas with the exception of *Neoagarddhiella baileyi, Pterocladiella capillacea* and *Ulva lactuca*. *Hypnea johnstonii*, which has been previously reported as a major food item in green turtle diet (López-Mendilaharsu et al., 2005), was available in the bay but not found in the stomachs of the turtles. *Gracilaria vermiculophylla* was present in 60%of the turtle stomachs analyzed and made up the greatest total percent volume (36%). Gracilaria textorii was present in the second greatest percent volume (16.5%).

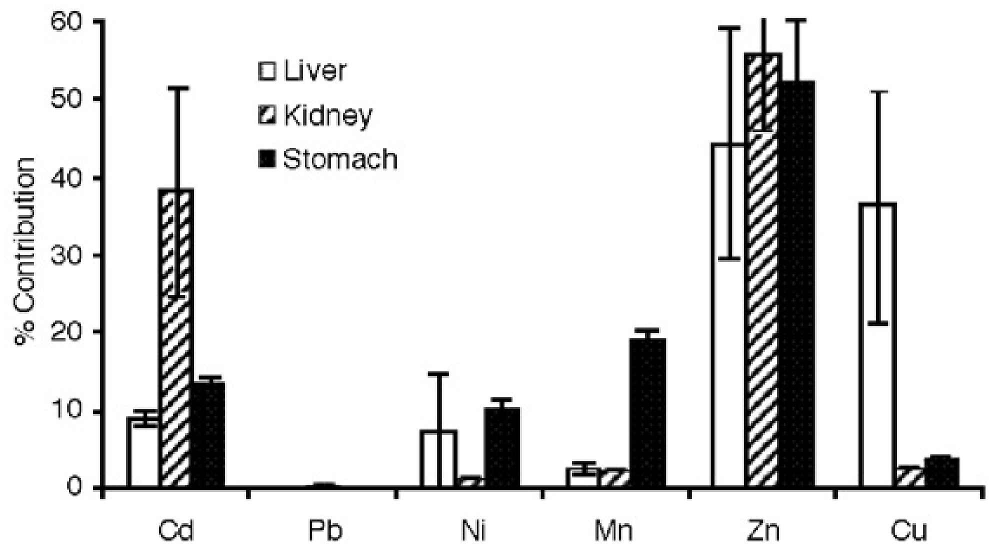

Fig. 7. Percent contribution of metals in tissues and the stomach contents of green turtles (*Chelonia mydas*) from Magdalena Bay, Mexico.

	Stomach Contents					
Species	1	2	3	4	5	TOTAL
Codium amplivesiculatum	69.1%					13.8%
Gracilaria textorii	30.9%	51.6%				16.5%
Gracilaria vermiculophylla		48.4%	33.6%		100%	36.4%
Neoagarddhiella baileyi				36.2%		7.2%
Pterocladiella capillacea				20.5%		4.1%
Rupia maritima				43.3%		8.7%
Ulva lactuca			31.8%			6.4%
Zostera marina			34.5%			6.9%

Table 3. Percent volume of macroalgae and sea grasses in the stomach contents of five green turtles (*Chelonia mydas*) collected in Estero Banderitas, Magdalena Bay, Mexico.

3.3 Principal components analysis

Principal components analysis (PCA) of the percent contribution of individual metals to the overall metal signature of each plant or tissue sample generated three principal components (PC) that explained 80.7% of the total variance in the data (50.1%, 17.6%, and 13.1%, respectively) (Fig. 8). Plots of the sample scores on the first and second principal components produced four groupings. Bay and stomach plant samples were separated by their scores on PC(1), while kidney and liver samples were separated by their scores on PC(2) (Fig. 8A). All but one of the bay plant samples obtained negative scores on PC(1), whereas plants from the stomach contents generally scored greater than 0. The loadings plot, which illustrates the influence of each metal on sample scores, indicated that the bay and stomach samples separated on PC(1) based on the dominance of the stomach samples' metal signatures by Zinc and Cadmium. The separation of liver and kidney samples appeared to be influenced by the greater contribution of Cadmium to the metal profile in kidney, and the dominance of Cu in liver samples which scored higher on PC(2) (Fig. 8B).

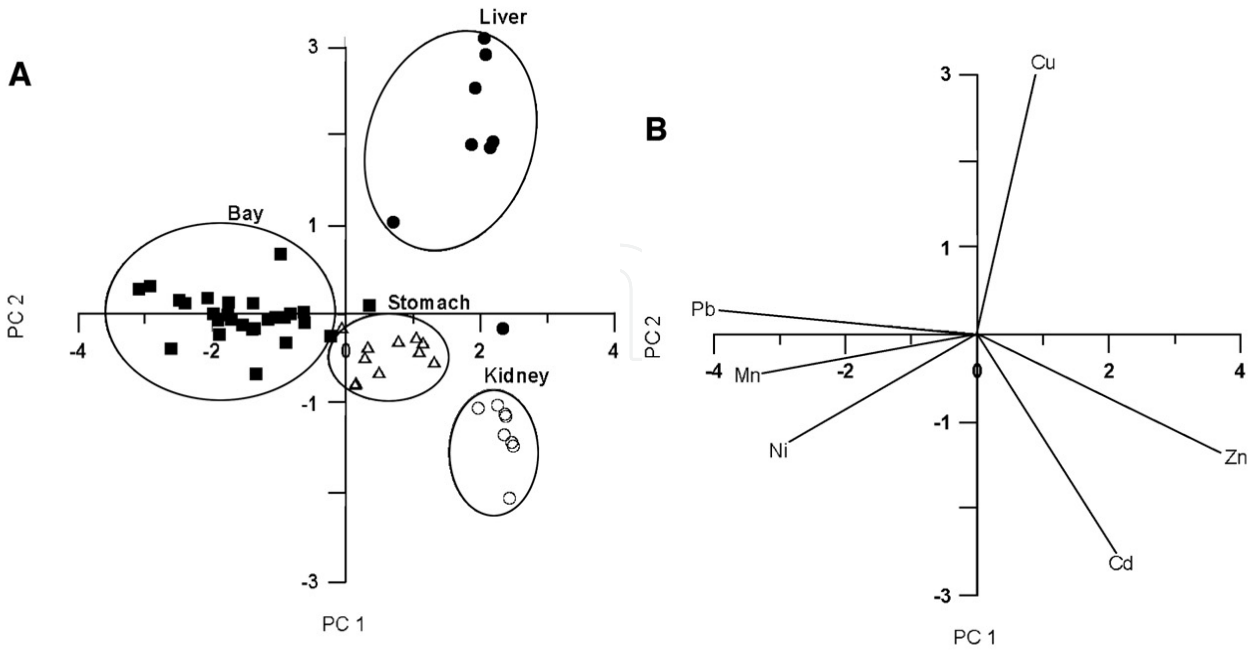

Fig. 8. A) Plot of sample scores of a Principle Component Analysis of the percent contribution of individual metals to the overall signature of marine flora from Magdalena Bay, kidney, liver and stomach contents samples from green turtles (*Chelonia mydas*). B) Loadings plot

3.4 Prescence of fibropapiloms and epibionts

In our study we found less of 1% of the animals with some fibropapiloms but at least 20% with epibionts. This is consistent with the data from Caribbean populations in where 2% had tumors present. The main observed difference was the degree of development between the populations in Bahía Magdalena (Fig. 9a) with very few in the carapace, frontal fins and head. In Caribbean populations bibber tumors (Fig. 9b) were observed and more concentrated in the head or in the frontal fins.

Fig. 9. A) Population in Bahía Magdalena (Fig. 9a) with very few in the carapace, frontal fins and head. B) Caribbean population's bibber tumors were observed and more concentrated in the head or in the frontal fins.

4. Discussion

Much of the literature on sea turtles has worked with absolute concentrations of metals, which is appropriate for comparisons of very similar sample types such as different sea turtle tissues or the same tissue in different sea turtle species. In the present paper we used absolute concentrations to compare metals in tissues (kidney vs. liver) and to compare metal concentrations in different plant species. However in order to better understand the source of metals to turtles in this region, the profile of all metals combined was used as an environmentally acquired marker. For this objective, we removed the influence of concentration differences among samples by converting the data to percent contribution of each metal to the total metal signature of the individual sample. This approach enabled the comparison of metal profiles across greatly different samples and was more appropriate than comparisons of absolute concentrations alone. For example, a single plant species located in two different areas will accumulate metals using the same physiological mechanisms. Therefore, a difference in metal profiles of the plant species from two different locations is an indication of differences in the availability of the metals from the environment. However, differences in the absolute concentrations of metals in plants would not necessarily indicate environmental difference because other factors might also be at play (e.g. age of the plant).

4.1 Comparison of metals in marine plant species

Metal concentrations in marine flora are controlled by both the bioavailability of metals in the surrounding water and the uptake capacity of the particular plant species. Marine algae have the capacity to accumulate trace metals several thousand times higher than the concentration in seawater (Bryan and Langston, 1992; Sánchez-Rodríguez et al., 2001). Red algae, such as Gracilaria sp tend to reflect the environmental availability of metals but have higher bioaccumulation of Cadmium, Copper and Zinc than other macroalgal groups (Sánchez-Rodríguez et al., 2001; Roncarati, 2003). These same species are a major component of the green turtle diet along the Baja California Peninsula (Seminoff et al., 2002; López-Mendilaharsu et al., 2005), and we proposed previously (Gardner et al., 2006) that their foraging habits could account for the high metal concentrations found in this population. However, comparisons across plant species in the present study suggest that species differences in metal concentrations are minimal. The only significant difference detected between plant species was that Cadmium was higher in *Ruppia maritima* than all other species, and higher in *Gracilaria textorii* than *Codium amplivesiculatum*. *R. maritima* was encountered in only one of the sea turtle stomachs analyzed, contributing a relatively small percentage of the overall diet (8.7%) in this study, and was absent from the diet of 24 green turtles analyzed in previous work (López-Mendilaharsu et al., 2005). *Gracilaria textorii* made up a larger proportion of the turtles' stomach contents (16.5%), but was similar in Cadmium concentration to most other plant species ingested by the green turtles. The results of the PCA also support this conclusion since the bay-collected plant samples grouped separately from the samples in the stomach contents despite that both groups consisted of the same five plant species.

We found significant spatial and temporal variations in heavy metal concentrations in marine plants as previous spatial studies has shown in the region (Páez-Osuna et al., 2000; Sánchez-Rodríguez et al., 2001; Rodriguez-Castañeda et al., 2006, Rodríguez-Meza et al., 2008). The high concentration of Zinc and Fe in the upper region might be related to the isolation of the site (Rodríguez-Meza et al., 2008). Heavy metal concentration was, in some

cases, in the levels of toxicity. Temporal variations in metal concentrations, such as high concentrations in Cadmium and other metals observed in April, may be related to local upwelling events. Surface water Cadmium concentrations have been strongly correlated with upwelling (Lares et al. 2002) which occurs during spring and early summer off the coast of Magdalena Bay (Zaytsev et al., 2003). These levels of Cadmium in seaweeds has not been observed in the Gulf of California studied populations but strong species and spatial variations where observed (Páez-Osuna et al., 2000; Sánchez-Rodríguez et al. 2001; Rodriguez-Castañeda et al., 2006). The differences in heavy metal concentrations that we found in the seaweeds did not generally correspond with patterns of those elements previously observed in the sediment from the same region or seaweed species (Rodríguez-Meza et al., 2008), contrary to the studied sites in the Gulf of California near a mine (Rodriguez-Castañeda et al., 2006) or near industrial ports (Páez-Osuna et al., 2000; Sánchez-Rodríguez et al., 2001; Rodriguez-Castañeda et al., 2006). This finding, together with the observed species differences, suggests that the metabolic condition and life cycle stage of the individual species might influence metal uptake and accumulation (Lobban and Wynne 1981). Similarly, Riget et al., (1995) found differences between seaweed species *Ascophyllum nodosum, Fucus vesiculosus*, and *Fucus distichus*. We found lower levels of Ni and Zinc in *H. johnstonii* than in the environment as reported by Rodríguez-Meza et al., (2008). Based on our data, there are similarities between the composition and concentration of heavy metals between the plant species reviewed and the sediment; except in the case of Cu, Fe, and Mn (Rodríguez-Meza et al., 2008). All those elements are considered critical in the photosynthetic metabolism (Lobban and Wynne, 1981). We might assume that those elements are more easily assimilated by the plants because of their use in photosynthesis.
The role of seaweeds and seagrasses in coastal lagoons (like Banderitas or any other along the Baja California Peninsula) are relevant because they are feeding grounds for black turtles (*C. mydas*), loggerhead turtles (*Caretta caretta*), olive Ridley turtles (*Lepidochelys olivacea*), and hawksbill turtles (*Eretmochelys imbricata*) and migratory birds like Brant geese (*Branta bernicla*; Seminoff, 2000; Herzog and Sedinger, 2004). All of the species are included in the Mexican endangered species list (NOM ECOL 059) and on the red list in the UICN endangered species (www. uicnredlist.org). They are high productivity areas for fishing all kind of products (CONABIO, 2000; Carta Nacional, 2005). The fact that we found more significant variation in the spatial than temporal heavy metal concentrations in most of the species show that they might be constantly incorporated in the diet of many herbivorous animals (Gardner et al., 2006) with severe consequences in their health. Management strategies for these species should consider monitoring the levels of metals.

4.2 Sea turtle tissue comparisons

Pb, Cu and Mn concentrations in tissue from this study were within the range of those reported for sea turtles in other parts of the world (Lam et al., 2004; Storelli and Marcotrigiano, 2003). However, the average concentrations of Cadmium, Zinc and Ni in kidney of green turtles from Magdalena Bay were high compared to previously reports for sea turtle tissues (Sakai et al., 1995, 2000; Storelli and Marcotrigiano, 2003). Studies of loggerhead turtles (Maffucci et al., 2005) suggest that sea turtles can regulate Copper and Zinc concentrations through homeostatic processes but that Cadmium uptake is not controlled by active process and thus tissue concentrations of this metal reflect exposure. In agreement with these findings, we observed that Cadmium concentrations in green turtle liver were similar to their food and that the Cu concentration in sea turtle liver was greater than in the stomach content. Similar relationships have been observed in green turtles from Japan (Anan et al., 2001). However, contrary to the findings of Maffucci et al., (2005), Zinc concentrations in the livers and kidneys of green turtles in our study were not significantly different from their stomach contents. The distribution of metals among organs is influenced by both duration and concentration of exposure. Liver is a major site of short-term Cadmium storage, whereas during long-term exposure, Cadmium is redistributed from the liver to the kidney where it is absorbed and concentrated (Thomas et al., 1994; Linder and Grillitsch, 2000; Rie et al., 2001). Therefore a significantly greater concentration of Cadmium in green turtle kidney than liver is often observed (Storelli and Marcotrigiano, 2003; Maffucci et al., 2005; Gardner et al., 2006) and likely results from years of accumulation in this long-lived species. While kidney Cadmium concentration may serve as a good indicator for assessments of sea turtle health, liver more closely reflects the concentration of this metal in the food and so analyses of liver may provide a better indication of recent environmental exposure. Accordingly, Cadmium concentrations in the livers analyzed in the present study were not different from the food in the sea turtles' stomachs. Concentrations of Fe and Zinc in liver were also similar to the stomach contents. Whereas, Plumb, Nickel and Manganese concentrations in liver were similar to kidney, but were lower than in the stomach contents, which may indicate metabolic processing of these metals. Alternatively, Copper concentration was higher in liver than in the turtles' food and appeared to be preferentially accumulated in liver over kidney.

4.3 Metals in sea turtle stomach contents and marine plants from the bay

Two principle components, PC(1) and PC(2), explained 68%of the total variance in the data.When plotted relative to PC(1) and PC(2), the plant samples collected in the bay formed a grouping at the left side of the plot while the green turtle tissue samples and the plants from the stomach contents plotted higher on PC(1) (Fig. 4A). Examination of the loadings plot for each of the metals confirmed that samples scoring high on PC1 had signatures dominated by Cadmium and Zinc (stomach contents and kidney) or Cu (liver) (Fig. 4B). This agrees with the observation that the plants in the stomach contents contained greater percent contributions of Cadmium and Zinc than the samples collected in the bay, while Pb and Mn contributed more to the metal profiles in the bay samples as shown in Fig. 2; a tendency that was consistent in all five plant species. The metal profiles in the sea turtle tissues more closely resembled the plants in the stomach contents than the same species of plants collected within Estero Banderitas. The fact that the concentrations of Cadmium, Fe and Zinc in green turtle liver were the same as the stomach contents but different from the plants collected in the bay suggests that sea turtles collected inside of Magdalena Bay use foraging resources outside of the Estero Banderitas region. Further support of this conclusion is provided by the fact that three algal species (*N. baileyi*, *P. capillacea* and *U. lactuca*) in the stomach contents were not found in Estero Banderitas. Franzellitti et al. (2004) proposed that tissue metal profiles can be used as "environmentally acquired markers" to determine sea turtle feeding areas. Similarly, principle component analyses have been applied previously to determine sources of metals in aquatic environments (Ruiz-Fernández et al., 2001). Comparison of the metal signature profiles in plants from the bay and the sea turtle stomach contents indicate that the plant species contained inside the sea turtle stomachs originated from a location outside of Estero Banderitas, in an area where Cadmium and Zinc concentrations dominate the metal profiles in the environment. Surface water metal concentrations have been strongly correlated with upwelling events and natural components of regional biogeochemistry (Daesslé et al., 2000; Lares et al., 2002). Similar to the distribution of nutrients in the water column, metals such as Cadmium and Zinc are depleted in the surface and enriched in deeper water. Upwelling processes are an important mechanism that brings elevated concentrations of both nutrients and metals to the surface and thus available for marine floral accumulation. Therefore it is highly probable that the sea turtles collected within Magdalena Bay are utilizing foraging areas in an upwelling-rich coastal region outside of the Bay. Coastal lagoons of the Baja California Peninsula such as Magdalena Bay have been identified as priority areas for sea turtle conservation programs (Nichols et al., 2000). Long-term sea turtle monitoring studies have demonstrated high site fidelity to Estero Banderitas over time, and low emigration of sea turtles from Magdalena Bay to other coastal lagoons along the Baja California Peninsula (Grupo Tortuguero, unpublished data). Efforts to protect areas within Magdalena Bay have focused on the creation of a refuge in the mangrove channels of Estero Banderitas, in part, because of the perceived importance of this habitat for sea turtle foraging (Nichols and Arcas, 2001). However, data generated by our work suggest that sea turtles residing in Estero Banderitas are feeding in areas outside of the bay, most likely in coastal regions with high upwelling. These findings support those of López-Mendilaharsu et al. (2005) and indicate that green turtles utilize spatially distinct feeding habitats within coastal areas. Therefore, we recommend that sea turtle protected areas be designed with an appreciation of regional rather than local scales in order to protect broader foraging areas.

4.4 Fibropapiloms and epibionts

The prescence of fibropapiloms are variable from 1.4% up to 90% of the population (Herbs *et al.,* 1999, Quackenbush et al., 2001, Chaloupka et al., 2009). The observed low proportion of the green turtles in Bahía Magdalena (less than 1%) agree with a well preserved environment and less stress situation for the animals. In the case of the epibionts we found a continuously prescence of cirripedia and balanus but not a diverse fauna like in the Atlantic that even polychaetes has been reported (Lara Uc, 2011).

5. Conclusions

Conservation of threatened species, such as the green turtle (*Chelonia mydas*), is closely related to habitat quality. In particular there are issues related to heavy metals, the presence of epibionts, parasites and fibropapiloms who might play a crucial role in the species survivorship. The process of metal bioaccumulation in marine food chains is poorly understood because very little data is available on metal concentration at different trophic levels and their temporal or spatial variation and its influence in turtle health. The Baja California Peninsula, Mexico serves an important role for feeding and developing sea turtles. High concentrations of metals detected in food items (seaweeds and seagrasses) and in green turtles (*Chelonia mydas*) from Magdalena Bay prompted an investigation into the sources of metals in the region in relation to the health issues of the animals. We compared metal concentrations in sea turtle tissues in relation to plant species found in their stomach contents, and with the same species of plants collected inside a sea turtle refuge area known as Estero Banderitas and determine the health state of turtles based on our long term monitoring efforts. Our results showed that Iron, Copper, and Manganese were the most significant metals found in seagrasses, red, and green algae. We found significant more variation in temporal heavy metal concentrations in relation to the maximum abundance in the samples and spatial variation in relation to the studied taxa suggesting that herbivores' have a differential intake of the metals. Also, our results suggest that heavy metals might be incorporated regularly in the diet of many herbivorous animals with severe consequences to their health. Differences in the metal concentrations between marine plant species in relation to animal tissue were minimal. Principal components analysis of the percent contribution of individual metals to the overall metal signature of each plant or tissue sample generated three principal components that explained 80.7% of the total variance in the data. The plant samples collected within Estero Banderitas formed a separate grouping from the green turtle tissue samples and the plants from the stomach contents. The plants in the stomach contents contained greater percent contributions of Cadmium and Zinc than the plants collected inside the bay, while Plumb and Manganese contributed more to the metal profiles in the bay samples. The metal profiles in the sea turtle tissues more closely resembled the stomach contents than the same species of plants collected within Estero Banderitas, and suggest that sea turtles collected inside Magdalena Bay use foraging resources outside of the Estero Banderitas region. Green turtle from Estero Banderitas seems to be healthy at this stage in comparison with nesting areas in the Pacific and Atlantic of Mexico our data on fibropapilomas and epibionts strongly support this idea. Our data supports the suggestion that metal profiles can be used as "environmentally acquired markers" to improve our understanding of the extent of sea turtle foraging areas. Management strategies for these species should consider monitoring the levels of metals.

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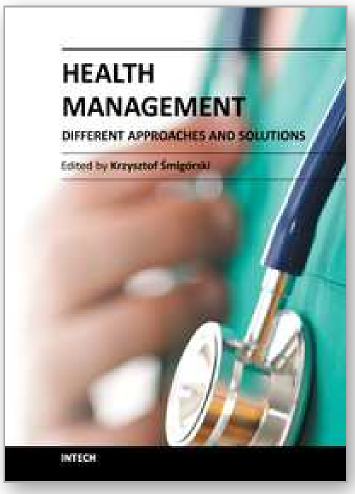

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The development in our understanding of health management ensures unprecedented possibilities in terms of explaining the causes of diseases and effective treatment. However, increased capabilities create new issues. Both, researchers and clinicians, as well as managers of healthcare units face new challenges: increasing validity and reliability of clinical trials, effectively distributing medical products, managing hospitals and clinics flexibly, and managing treatment processes efficiently. The aim of this book is to present issues relating to health management in a way that would be satisfying for academicians and practitioners. It is designed to be a forum for the experts in the thematic area to exchange viewpoints, and to present health management's state-of-art as a scientific and professional domain.

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